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Biochemistry

FOURTH EDITION

Lubert Stryer

Library of Congress Cataloging-in-Publication Data

Stryer, Lubert.
Biochemistry/Lubert Stryer.—4th ed.
Includes index.
ISBN 0-7167-2009-4
1. Biochemistry. I. Title.
QP514.2.S66 1995
574.19'2—dc20 94-22832

Printed in the United States of America

Fourth printing 1997, KP

What then determines whether a protein will adopt a globin fold? *The main-chain conformation appears to be specified by the pattern of nonpolar and polar residues.* Thirty-two positions in the sequence are nearly always hydrophobic; all these side chains are inside the protein. Surprisingly large changes in the volume of these residues can be tolerated. Helices can shift their position by several angstroms and their orientation by as much as 30 degrees to compensate for changes in the volume of the hydrophobic core. Another 32 positions are nearly always occupied by a charged or polar residue, or by Gly or Ala. These hydrophilic residues are located on the surface of the protein. *The distinctive hydrophilic versus hydrophobic pattern of these conserved residues, which comprise nearly half the total, distinguishes globins from all other proteins.*

Mutagenesis studies of λ repressor, a phage protein that controls gene expression (p. 959), were carried out to learn the range of allowable sequence changes in a functionally important region. A helix of one monomer packs against the corresponding helix of another monomer to form a dimer that binds to specific sites on DNA and silences gene expression (Figure 16-27A). Two or three residues at a time were simultaneously mutated by using mixtures of nucleotides to synthesize codons for all twenty amino acids at these positions. Functional proteins were then selected and sequenced. The important finding was that *most positions can be changed with retention of function* (Figure 16-27B). In particular, solvent-accessible residues can readily be substituted, whereas buried ones are much more conserved. For example, the activity of the repressor was unaffected by the replacement of an external glutamate with any of 12 other amino acids.

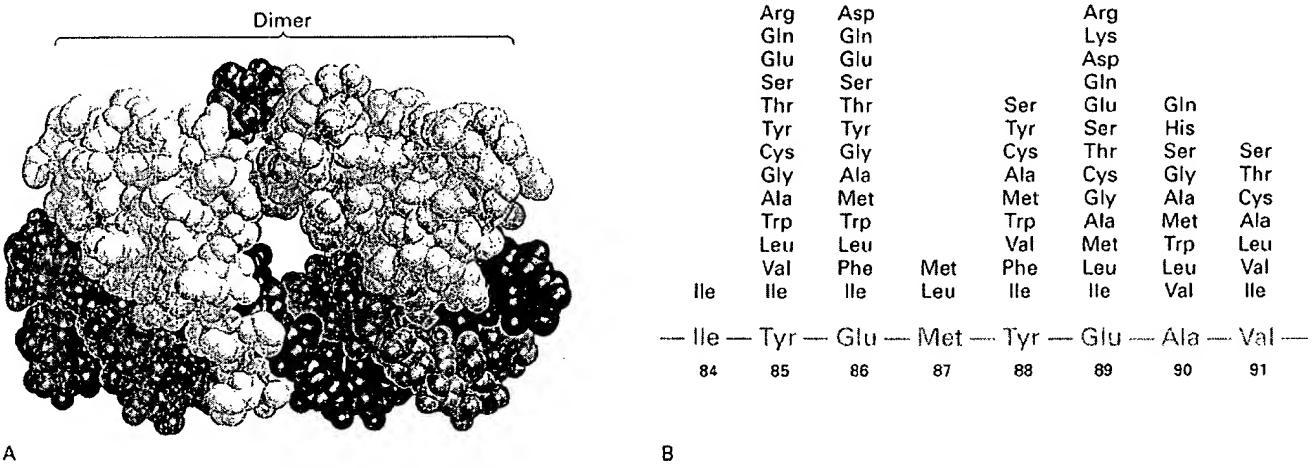

Figure 16-27
Structure and mutagenesis of the repressor protein of λ phage. (A) λ Repressor binds as a dimer (yellow and blue) to DNA (green and red). Helix 5 (shown in darker color) of one subunit interacts with helix 5 of the other subunit. (B) Functionally acceptable residues of a key part of helix 5, as determined by mutagenesis. [(A) Drawn from 1lmb.pdb. L.J. Beamer and C.O. Pabo. *J. Mol. Biol.* 227(1992):177. (B) After J.F. Reidhaar-Olson and R.T. Sauer. *Science* 241(1988)53.]

AN ENCOURAGING START HAS BEEN MADE IN PREDICTING THE THREE-DIMENSIONAL STRUCTURE OF PROTEINS

One of the goals of protein chemistry is to be able to predict the three-dimensional structure of a protein, given its amino acid sequence. Prediction is difficult because (1) a polypeptide chain has a vast number of potential conformations and (2) proteins are only marginally stable, which means that the free-energy difference between the unfolded and folded states is small. *No simple code relates the one-dimensional information of a sequence to the rich three-dimensional form of the folded protein.* The folding problem is inherently complex.

Significant progress is now being made in this challenging area of inquiry for several reasons. First, the structures of an increasingly large number of proteins are being solved by x-ray crystallography and NMR spectroscopy. More than 100 different protein folds are now known. Second, DNA sequencing is providing a wealth of amino acid sequence information. By comparing the sequences of homologous proteins, we can learn far more about the rules governing structure than from a single sequence. Third, sophisticated computer programs are taking advantage of the rapidly enlarging sequence and structure databases. Subtle patterns and relationships can be deduced and displayed. Fourth, predictions of structure can be rapidly tested. Theory and experiment have come together and are mutually reinforcing.

The first step in structure prediction is to ask whether the sequence of a new protein is similar to one whose three-dimensional structure is already known. *If two proteins are more than 40% identical in sequence, their backbone conformations are very likely to be nearly the same in the region of partial identity.* A pair of proteins differing markedly in sequence can have essentially the same backbone structure *if their hydrophobicity patterns are alike.* The structural similarity of root nodule leghemoglobin and human hemoglobin is a striking illustration (see Figure 16-26). *Functional motifs in proteins can also be identified by scanning amino acid sequences.* Calcium-binding EF hands (p. 348), for example, can be found by searching for a distinctive pattern of hydrophobic and oxygen-containing side chains in successive 29-residue segments of an amino acid sequence (Figure 16-28). X-ray crystallographic studies have shown that recoverin, a calcium sensor in vision, indeed contains EF hands (Figure 16-29), as was predicted from its amino acid sequence.

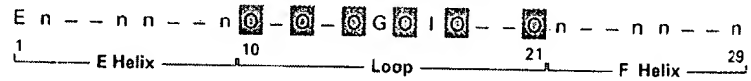

Figure 16-28
Consensus sequence for calcium-binding EF hands in proteins. EF hands can be detected by scanning amino acid sequences for this 29-residue motif. The symbol n (yellow) denotes a nonpolar side chain, and O (red) denotes an oxygen-containing side chain (Asp, Asn, Glu, Gln, Ser, Thr). The residues marked in red form the calcium-binding loop.

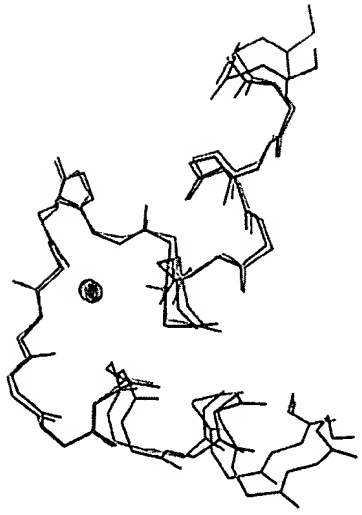

Figure 16-29
The EF hands of calmodulin (blue) and recoverin (red) are very similar in structure. Bound Ca^{2+} is shown as a sphere. The mean difference in the position of main-chain atoms is 0.8 Å. [Drawn from 3cln.pdb. Y.S. Babu, C.E. Bugg, and W.J. Cook. *J. Mol. Biol.* 204(1988):191; and 1rec.pdb. K.M. Flaherty, S. Zozulya, L. Stryer, and D.B. McKay. *Cell* 75(1993):709.]

Investigators are now tackling the most demanding problem, the prediction of structure in the absence of prior three-dimensional information about a related protein. By aligning many homologous sequences from different species, one can discern the pattern of essential hydrophobic and hydrophilic residues. This pattern is the starting point in identifying folding units. The accurate prediction of much of the backbone structure of the catalytic domain of protein kinase A before its crystal structure was solved is indicative of the rapid progress that is being made.

PROTEIN DESIGN TESTS OUR GRASP OF BASIC PRINCIPLES AND CREATES USEFUL NEW MOLECULES

The design and synthesis of novel proteins and of variations on nature's themes are important for several reasons:

1. De novo synthesis tests our understanding of fundamental principles. The ultimate criterion of the validity of a theory is its predictive